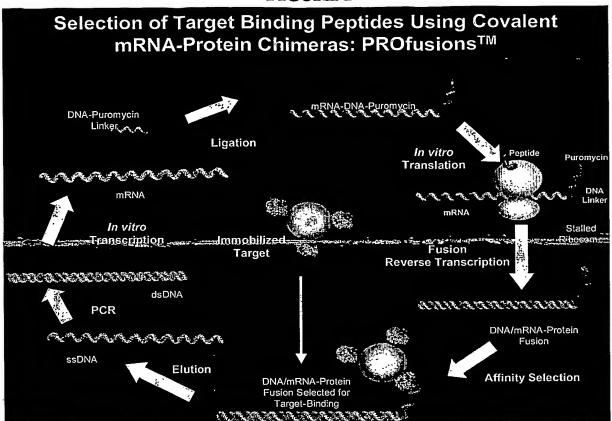
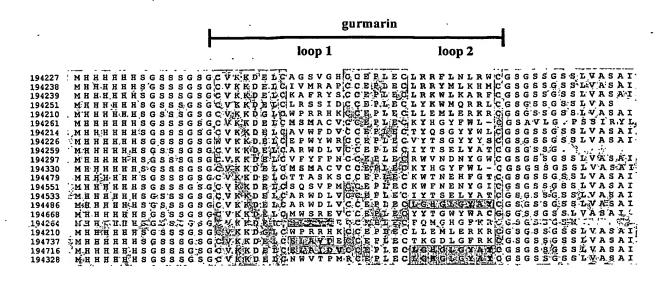


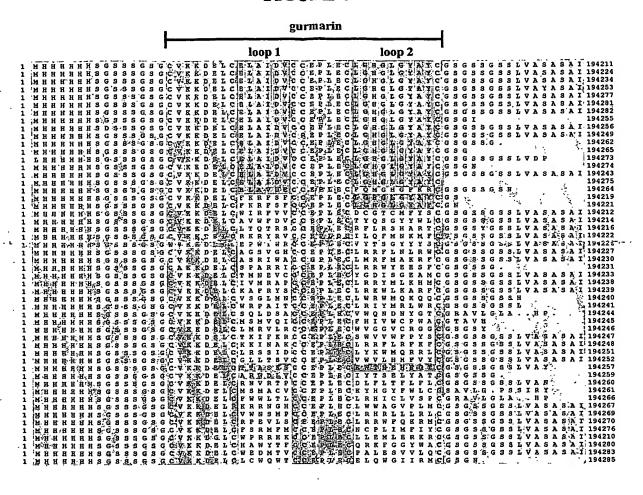
EEST AVAILABLE COPY

FIGURE 2



ă	Target: Pertussis toxin	Library: Gurmari	rin						
コ		1				Epoxy-PT	biot PT	biot PT	
		R	R2	R3	1 74 S	R5a	R5b	R6a	R6b
=	PCR VINE TO SERVE TO			· · · · · · · · · · · · · · · · · · ·	Agriculture Constitution of the Constitution o	11、11、11、11、11、11、11、11、11、11、11、11、11、		from R5a	from R5b
	1.1. Analytical RT-PCR		-						
1	specific signal after x rounds of PCR		18	24	24	24		24	15
Ï			21	27	24			27	24
1	1.2 Preparative PCR	,	是 14 14 14 14 15 15 15 15 15 15 15 15 15 15 15 15 15	まずのは、少さいので	The state of the s	11、11、12、12、12、12、12、12、12、12、12、12、12、1	3.		
•	done With 2 rounds of PCK		36	30	30	32		90	30
i	scale		2x 500 ul	1×500 id	1 500 11	1×500 11		12 500 11	4× 600 m
İ	DNace direction dane		200	1 000 VI	יין ממני עי	וא סחני או		IN DOC XI	IT ONG YI
တ	NAP5 gelfitration		OII	New York Williams	) Aes	yes		yes	yes
	Inp/E1/E2/E3		500/300/400/200	500/300/400/200	500/300/400/200	500/300/400/200		500/300/400/200	500/300/400/200
	yield purified RNA in pooled E1 (OD260)		20 nmol	13 nmol	12.7 nmol	11.6 nmol		16.3 nmol	17.6 nmol
1	remaining material					. 6.6 пто			
₹		A CONTRACTOR OF THE SECOND		はない はなるのでは	18 A. C.				
1	Linker type		PEG2A18	PEG2	PEG2	PEG2	. 427.	PEG2	PEG2
土	Coupling efficiency		O DIMOI	2 nmoi	5 nmol	5 nmol		5 nmol	5 nmol
	ivield linker counied RNA		4.2 nmol	2 5 pmol	2 6 000	2 6 000		70%	70%
u	In with Translation and DDAftestantit.			7 V.	3.3 111101	9.0 111101		3.5 mmol	3.5 nmoi
5	formation						1		
	input		4.2 nmol	3.5 nmol	3 nmol	3 nmol		3 nmol	3 nmol
	RNA / 200 µl lysate		260 pmol	290 pmol	250 pmol	250 pmol		250 pmol	/250 pmol 250
	salt incubation at -20°C		over night	over night	over night	· over night	120	over night	١.,
ဗ	100 A 40 A 40 A 10 A 10 A 10 A 10 A 10 A	当年のように関われます。	れている。	1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1	10000 100000 100000 100000 100000 100000 100000				
1	purified on x coloumns		7	4	4	4		4	4
1	efficiency		4.5%	4.4%	2.7%	7.2%		3.3%	2.7%
ŀ	yieid		187 pmol	156 pmol	81.1 pmol	· 217 pmol	A. 1877	97.6 pmol	81.1 pmol
1	Keverse transcription	10年のでするとです。	を できる できる のの できる	では、一般などのでは、	ないないない なるがら	はいるというないと			
1	conditions		1 mM DTT	1 mM DTT	1 mM DTT	1 mM DTT		1 mM DTT	1 mM DTT
•			168 pmol (3/4)	72.6 pmol (1/2)	50.4 pmol (3/4)	50 pmol		40 pmol	50 pmol
ö	nis-1ag puritication	The second secon			The second second				
	upor		168 pmol	90.75 pmol (72 pmol (67 pmol (72 pmol (97 pmol (97 pmol ohne RT) (97 pmol ohne RT)	61,9 pmol (3/4 RT + 1/4 OdT	45 pmol RT + 27.6 pmol RNA		35 pmol RT + 15 pmol	43 pmol RT + 19
	efficiency	34%			43%	41%		36%	43%
1	yield	55 pmol	66.6 pmol	59.2 pmol	26,4 pmol	27.6 pmol		18 pmol	26.4 pmol
寸	oţ		450 րվ	450 µl	450 µl	450 µl		450 µl	450 µl
ام	selection	Mary Mary Mary Control	では、大学の大学では、	· · · · · · · · · · · · · · · · · · ·	The State of the State of	Seminary of the service of the servi	4.		
1	selection volume	Im.	- E	1 m	-  E	1ml	1m1	1ml	1 mi
$\pm$	finduction of immigrant	18 pmoi	5 pmol	5 pmol	5 pmol	5 pmol	5 pmol	5 pmol	5 pmol
#	milai Concentration of immigazor	45 MM	е тм	Mm 9	8 mM	11.4 mM	11.4 mM	18.8 mM	21.5 mM
_		ox 100 pr mbs blocked streptavidin		3X 100 µ HBS 3X 100 µ HBS olocked streptavidin blocked enoxy beads	3x 100 µl HBS	2x 100 µl HBS blocked enoxy	3x 100 µl HBS	3x 100 µf HBS	3x 100 µl HBS
		peads			saturated	beads	saturated	saturated	saturated
					streptavidin beads		streptavidin beads	streptavidin	streptavidin
Ц	first preclear binding	%0	background	background	background	23/24 dpm	80/50/32 dpm	37/33/34 dpm	72/30/37 dom
	beads saturated with a puls of biotin	yes	yes	2	ves	2	ves	Sex	Nas
	effective concentration of PT	100 nM (50 nM)	100 nM (50 nM)	50 nM	100 nM (50 nM)	50 nM	100 nM (50 nM)	100 nM (50 nM)	100 nM (50 nM)
1	conditions				60 min at RT	90 min at RT	90 min at RT	90 min at RT	90 min at RT
$\exists$	specific binding %	•	•	1	0.2%	1.0%	5.2%	9.4%	11.6%





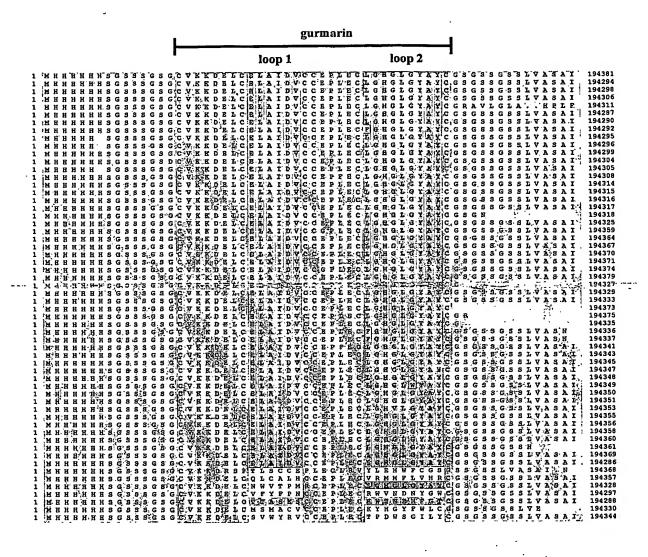


FIGURE 5

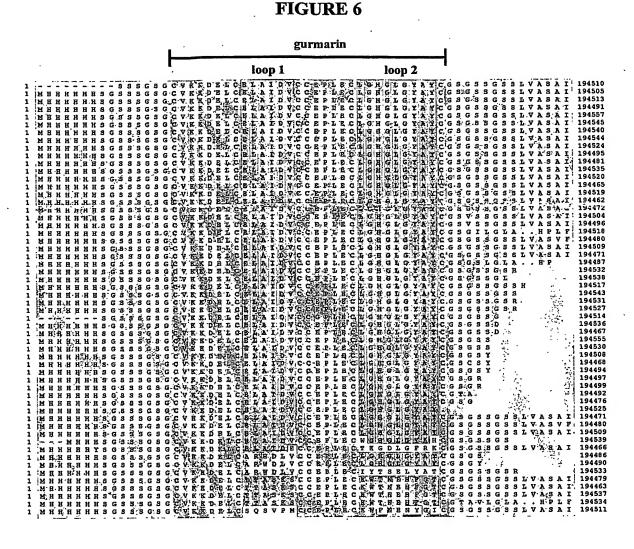
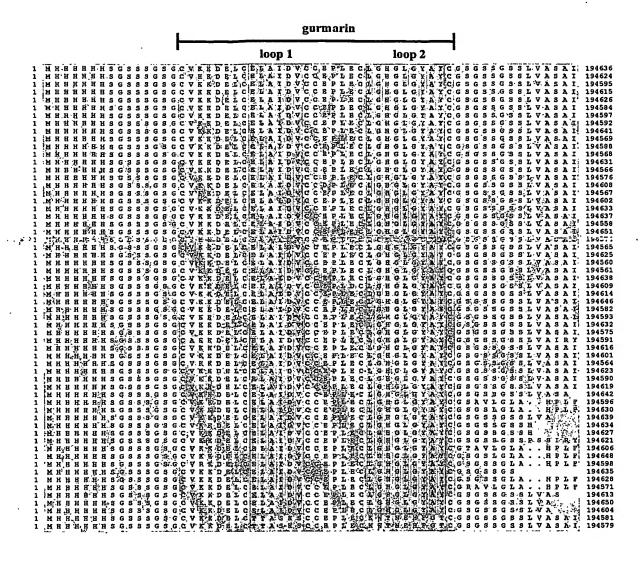


FIGURE 7



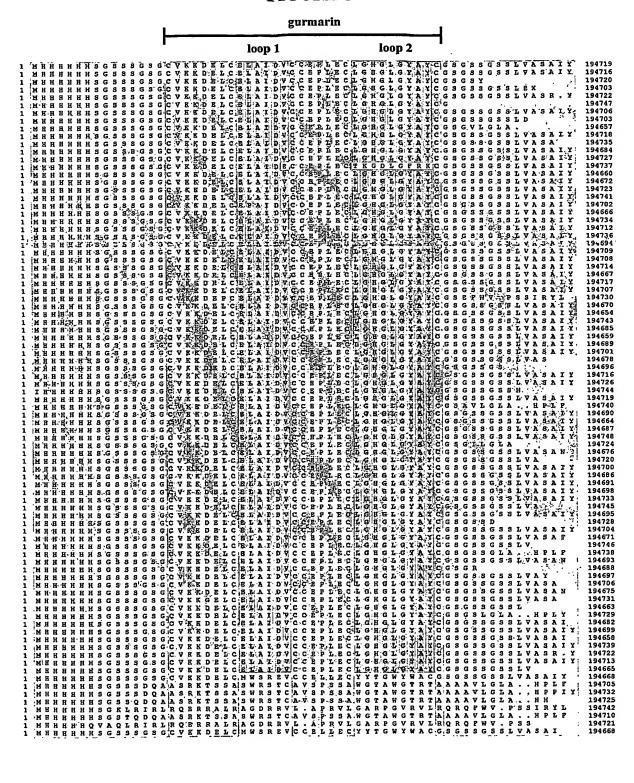
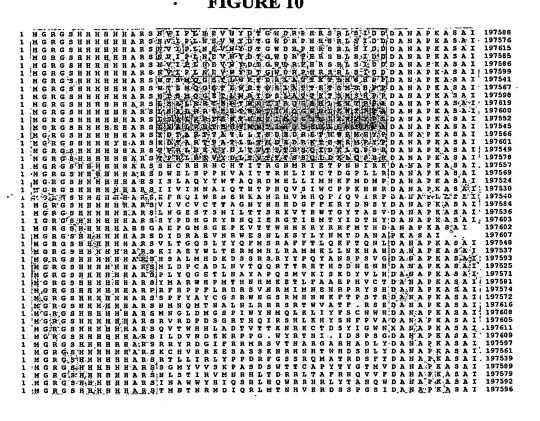
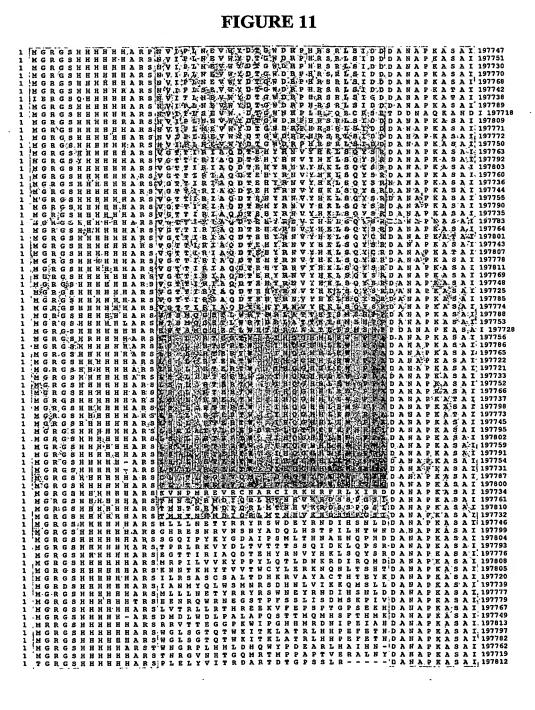


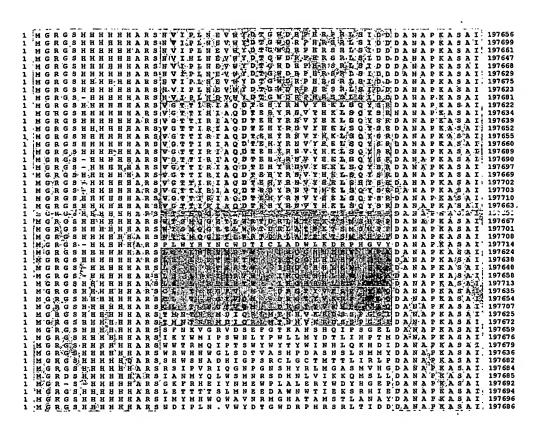
Table 2: selection of exists of PERS selection against immobilized PT

<u>, , , , , , , , , , , , , , , , , , , </u>				_		TO mai hood wood	5		
		The second secon		M. Miller	Marie Commended and Marie College Commended and the leading of the London College Coll	έH	DIOLE	THE PARTY AND ADDRESS OF	Diot P.
		R	<b>6 6 1 7 3</b>	83	<b>7.</b>	RG	R5D		KGD
1	PCR		The state of the s	· 医神经检验的 (1981)				from R5a	from R5b
	1.1. Analytical RT-PCR						The state of the s		
_	specific signal after x rounds of PCR		18	. 81	24	,			
	control signal after y rounds of PCR		21	24	24	24		A STATE OF THE PROPERTY OF THE	المعموم الاستانية مواصية مقائد المرات
1.2	2 Preparative PCR								STATE OF THE PARTY
$\exists$	done with z rounds of PCR		38	30	30		Actual Control of the		
	quality			single band	single band	single band	100 miles	į	
2.	In who transcription			Ben Samuel	いのでは、				74.73 A
	scale		2x 500 µ	1x 500 h	1× 500 µ	1x 500 L			
	DNase digestion done		29	00	yes	sax	2. F. F. C.		
									***************************************
97	NAPS gelftration	1000年の日本の日本の日本の日本の日本の日本の日本の日本の日本の日本の日本の日本の日本の							1人人
L					500/300/400/200	500/300/400/200			
L	yield purified RNA in pooled E2 (OD260)		28 nmol	16 nmol	8.3 nmol	12.4 nmol	processing and a second		
	Market Committee of the							The state of the s	
L				PEG2	PEG2	PEG2	The state of the s		
£	ionii		8 nmol	L	5 nmol	5 nmol	115年11年11年11年11年11日		
1	Septimental Control of the Control o		70%	%UZ	70%	%0Z	The Part of the Pa		
$\pm$	Compining entremely		42 pmg	2 46 mmol	3.5 pmol	3.5 nmol	The state of the s		
$\frac{1}{2}$	yield linker coupled Kilwa		17.4 IIIIO	O.13 IIIIQ		0111100			
-	Angual	A STATE OF THE PARTY OF THE PAR	The Call of the Ca		Control of the State of the Sta		100		DEBEST OF STREET
o	In Vide Hanslauon and rusagen tonnauon.	The Property of the Party of th		200	37.7	TO SEE STANKING TO SEE STANKIN			
$\frac{1}{2}$	Indui		4.2 nmol	3.15 nma	Sumo	Sumo	は ない		
$\frac{1}{2}$	RNA / 200 p		Z60 pmol	260 pmol	юша ост	iomq ucz			
	salt incubation at -20C	over week	over night	over night	over night	over right			ية الله و المالية الما
9		をよくし、これを見ることを		からなるというとは、	SECTION OF THE SECTIO			100	
	purified on x coloumns		2	4	4	4			
1	efficiency	8%	2.1%	1.89%		5.8%	7. 1.		
1	100	The second section of the second	8	Se./ pmol	omg e, ea	1/o pitto	The state of the s	J. Committee of the Com	The same of the sa
2	Reverse transcription			いるかが、大きな人		が見るという。		Cond. of Fig STREET, C. S.	The second secon
	conditions		1 mM DTT	T	1 mM DTT	1 mM DTT	2.4.4		
	input		66 pmol (3/4)	42.5 pmol (3/4)	40.3 pmol (3/4)	50 pmol	Y Comments of the Comments of		
_	efficiency of RT						The state of the s		
	yield of RT-RNA					,	が対象を		
	portion of reverse transcribed Fusagens								
8.	His-Tag purification on Ni-NIA agarose							The second secon	1000円の行政に対象が
	conditions: endconcentration of DTT from RT		0.75 mM	0.7 mM	0,52 mM	, 0.5 mM	D. W. S. W. S.		
				42.5 pmol (RT Ansatz	47 omol (3/4 RT + 1/4	45 pmol RT + 21 pmol			
	Input		88 pmol	ohne nicht-RT)	OdT				
L	efficiency		72%	30.6%	35%	41%			
$\frac{1}{2}$	yield	55 pmol (1/4)	19.7 pmol	12.2 pmol	18.5 pmol	27.6 pmol	は、 は		
1	in volume of	1	450 µ	300 п	450 µ	450 µ			
1.0		Oligo(d1) puntication	in the second se	CONTRACTOR ACCUSATION	Trees that are	THE PARTY OF CASE WITH			The state of the s
	solodion volume	1_	Imt	1 ml	1 ml	Im!		1	1
l	Paris Comments	18 nmol	5 pmol	53 nmol	5 pmol				
1	final concentration of immidazol	25 mM	18 mM	19.5 mM	6 mM	12,3 mM	3v 154 mM	3v 426mpM	3x34B pmM
1							blocked, blotin	blocked, biotin	blocked, blotin
	predear	5x 100 Pastreplayidin beads	5x 100 Fill streptavidin beads	3x 100 pl epoxy beads	-	2x 100 Fl	<b>75</b>	saturated streptavidin beads	saturated streptavidin beads
	first preclear binding %	%0	0.5%	background	0.5%	. 42 dpm	$\perp$	278/214/208 dpm	72/30/37 dpm
L	selection on biotinylated Pertussis toxin (1944)	serie 1	serie 1		serie 1		serie 1	serie 1	serie 1
	Epoxy-bead coupled PT			serie 1		serie 2			
	PT- beads saturated with a puls of blotin	yes	yes	yes	yes	2	yes	yes	yes
$\frac{1}{2}$	effective concentration of PT	50 nM (1-1)	20 nM (***)	50 nM (12.5)	SO nim	50 nM	100 nM (50 nM)	100 nm (50 nm)	100 nm (50 nm)
1	conditions	over right at 4C	60 min at RT	90 min at RT	60 min at RI	90 min at Ki	SO MIN BILK!	SO WITH BILK!	SU min at Ki
	specific binding %	•		•	1.2%	%¢r0	4.2%	6.0.0	5,078

197588	M	R	G	s	н	H	Н	H	H	H F	Ŕ	s	Ń	V.	i	P :	L J	N I	3.1	7 W	Ý	Ď	Ţ	Ġ	W:	O F	ĹĘ	H	k	S	R	Ļ	Į	p	D	D.	A	N.	A. I	PF	K A	5	A	I
197947	. M C	R	G	s	H	H	H	H	H	H Z	R	S	Ÿ	G	Ť	T	1	Ŗ	7	Q	D	T	E	H	Y 1	R N	V	X	H	K,	L	S	2 X	s	R	D.	A I	N.	A 1	9 I	KA	S	A	Į.,
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198000	MG	R	G	S	Н	н	Н	H	Н	H P	R	s	W	R	D	T_	R 1	K J	. I	I M	R	H	Y	F,	P 1	L 2	\ I	ם	s	Y	W	ĎΙ	T	P	R	D.	A I	N.	A :	PI	K P	. S	A	I;
197983	M G	; R	G	S	H	H	H	H	H	H P	R	S	12	T	\$	1	<b>Q</b>	3.1	181	χĹ	1	·R	14:	D.	B,:	$C_{\mathbf{k}}$	υŢ	,T	į K	41	S	<u> </u>	3 8	P		D.	A	N.	A :	₽ 1	K P	15	A	I
197998	MO	3 8	٠G	s	H	H	Н	Н	Ħ	H F	I A	R	s	P	L	W	Y I	H :	ľ	1 C	M	D	T	I	C :	L A	, E	W	L	ĸ	D	RI	В	G	V;	Y	D ?	A	N A	A I	PF	( A	. S	A ]
															-	- 197		DOTE:	-177	****				1217	TTP:	20713	27.7.2	-	ne os	7 4 TM	****	7412	בגזלי	a series										
197836	MO	3 1	G	S	н	H	H	Н	H	H Z	R	S		1		ZI :	n a	13	3 6	A .	1				D.	RH	<u> 111</u>	12	130	K,	R	P.	eV.		Q.	D	A	N.	A :	P 1	K P	ı s	A	I.
197552	' M (	3 F	G	S	Н	Н	Н	н	Н	H A	R	S		5	Ά,		L.	2	<u>)</u>	1	1	1.1											1.0			D	A.	N.	A :	PI	K	1 S	A	I
	•												,		<b>,</b> -						- 10						<del></del> .							<b>.</b> .										
197596	MO	F	G	5	H	H.	н	H	н	H 2	A.R	S	T	M	N.	T	N.	н.,	4.0	) · I	္ပစ္	R	L	M	T 1	N 1	١V	Ķ	R	,D	S	5.1	₹,G	S	.II	Þ	A	N.	A	P	K	1 S	A	I.
																											•									h		٠						•
197569	. M C	3 F	G	S	н	H	н	H	H	H Z	l R	S	D	W	E	L	s:	P	P	ΙV	Α	I	T	T	R :	H I	. 1	N	C	T	D	G I	P	· L	R	D	A	N.	A :	PΙ	KI	1 S	A	I
197536	ME	3 F	t G	S	Н	H	H	Н	H	H A	\ R	S	L	N	G	Е	s '	T :	S	1 1	L	T	T	s	R:	K 1	7 7	. E	W	T	G	Y ?	C A	S	v	D	A.	N.	A	P 1	K I	1 S	A	. I
				٠.					٠.				1																															
197611	M	j F	( G	S	H	H	H	H	H	H A	R	s	Q	V	T	W	H :	H	L 2	A D	T	v	T	Т	K	N F	R F	C	T	D.	·S	Y:	I G	W	N	X	A	N.	A	PI	K I	1 S	A	I.
197530	`M (	3 6	t G	S	H	H	H	Ħ	Ħ	H. I	l R	s	I	I	v	I	H	N A	A I	ΙQ	T	Н	T	P	H	Ω,	7 5	ï	W	С	P	P	K H	N	R	D	A.	N.	A	P :	K I	ı s	A	I.
197557	MO	3 F	t G	S	н	H	H	Н	H	H 2	\ R	S	s	H	С	R	H :	R I	N C	СН	T	Ι	T	R	G :	N F	1 F	ľ	E	Т	P	N	ı,	R	K	D	A.	N.	A	P	K Į	1 S	Α	Ϊ́
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197797	· M (	3 F	l G	s	Н	H	H	H	Н	H I	1.Ŗ	. \$	W	G	L	s	G '	T (	Q :	r W	K	Ι	T	K	L	A 7	r e	L	Н	Н	P	E I	FE	T	N	D	A	N.	A	P	K.Į	1 S	A	. I
	ī		•	٠.	.:.						;																									•	_		٠			4	5	
197888	ĽM (																																											
197897	M (	3 F	R G	Ş	H	H	H	H	Ħ	H I	L	L	W	G	P	₽	S	G :	S I	PR	T	R	S	T	T	G 2	r s	T	T	S	S	P	S I	. P	G	T	Ľ,	T	L.	R I	R J	1 2	Н	٠,
197825	M'C	3 E	\_G	S	H	H	H	H	Ĥ	Ħ,	A R	_5	W	Q	P	E	V	K I	M S	S S	L	v	D	T	S	Q	F٦	7 G	A	A	v	E :	r F	T	Т	D	A	Ŋ	A	P	K Z	7 S	Α	
	-	-																																										•







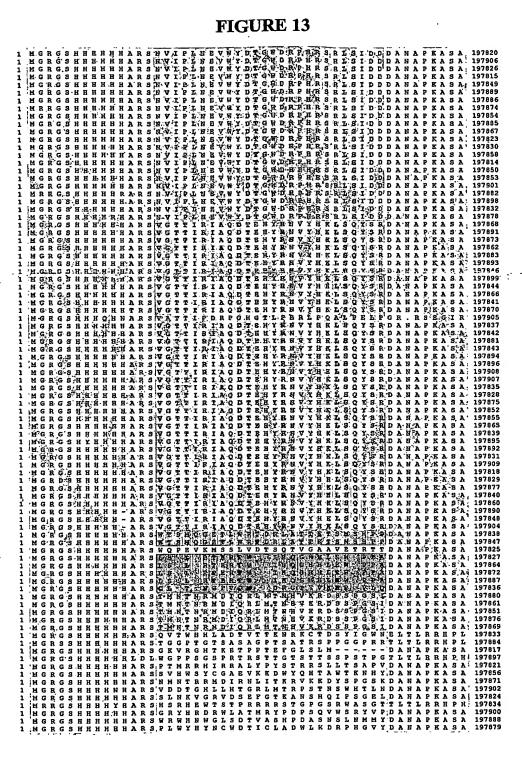
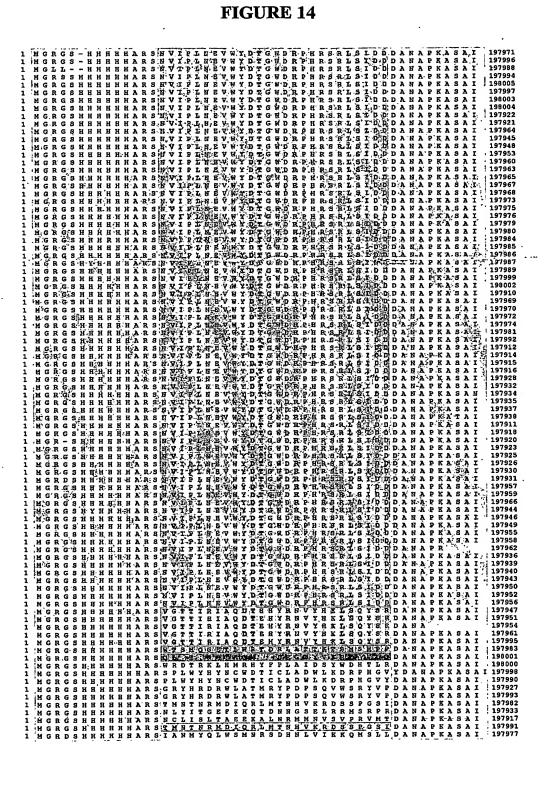
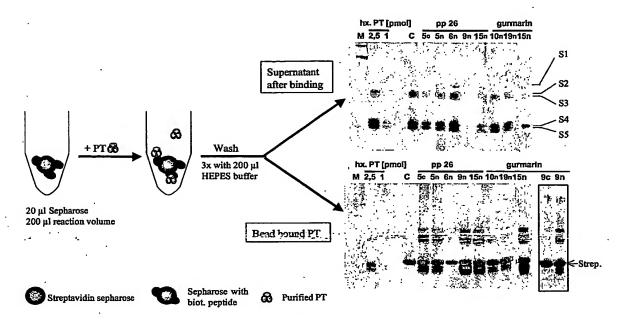
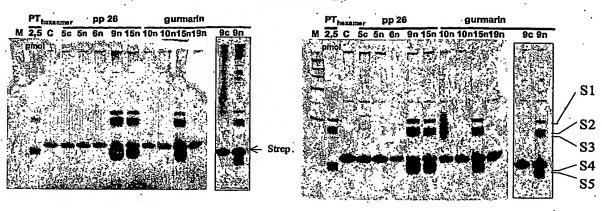


FIGURE 14

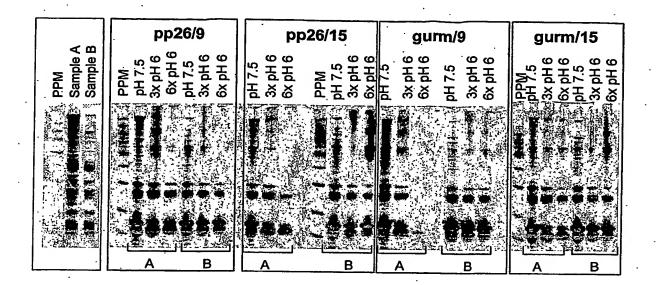




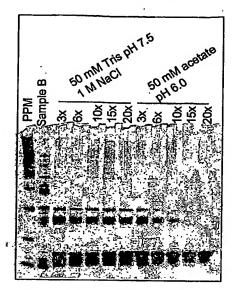
18/28 **FIGURE 16** 

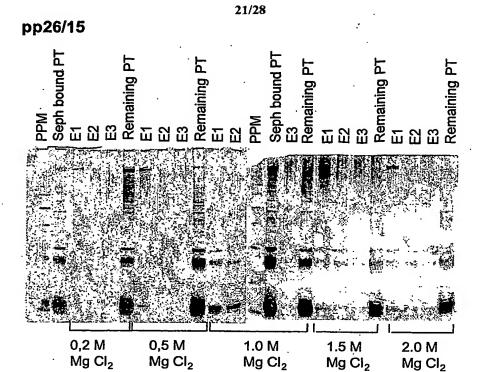


19/28 **FIGURE 17** 

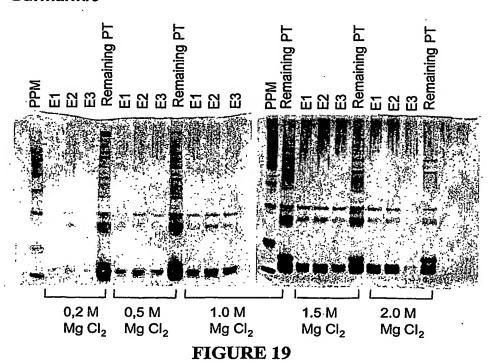


20/28 FIGURE 18.





## Gurmarin/9



Elution with glycin pH 2.5

Elution with carbonate pH 10.5

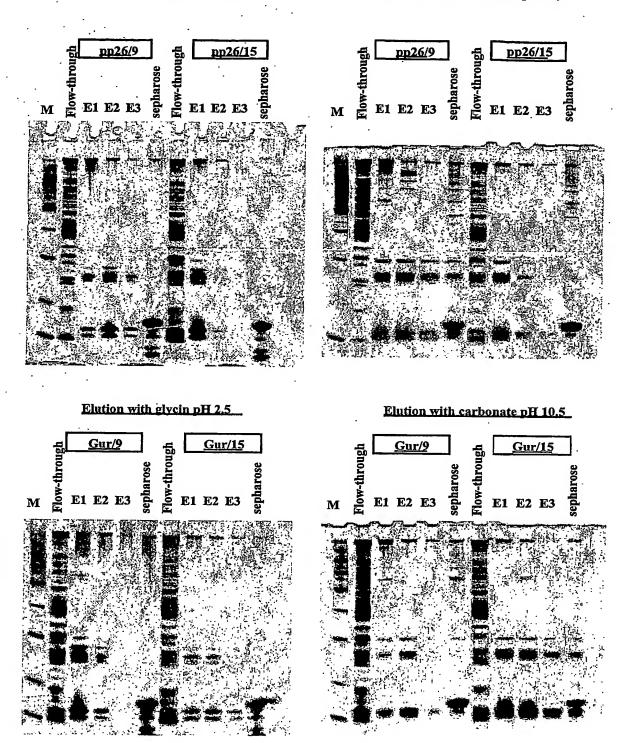


FIGURE 20

FIGURE 21

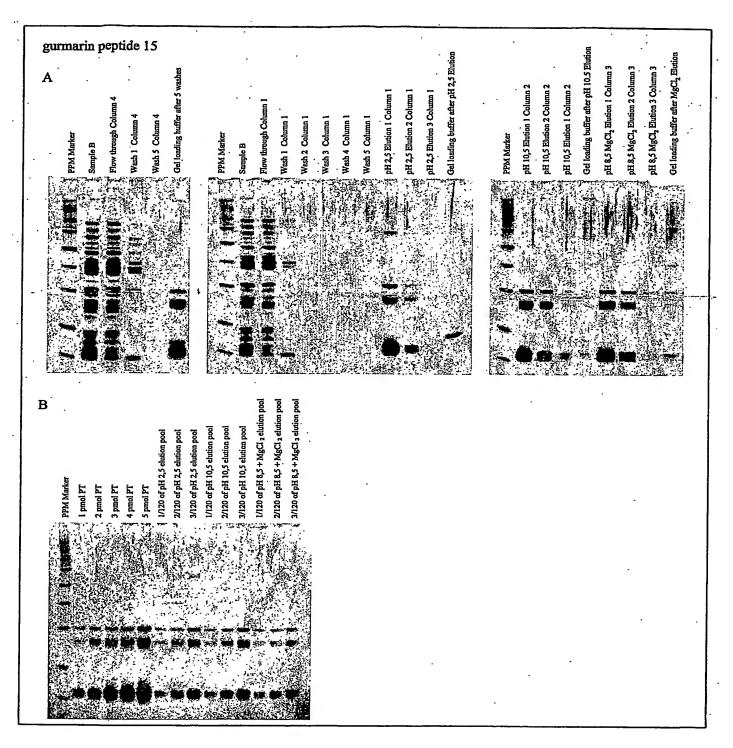
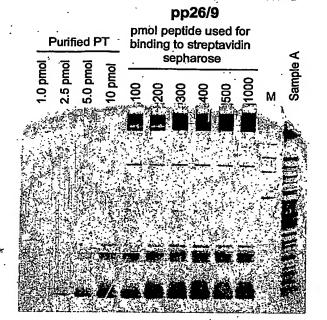
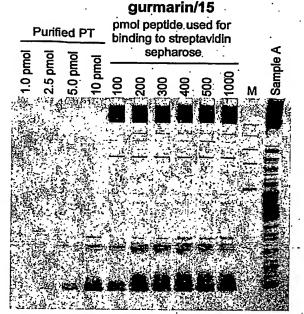
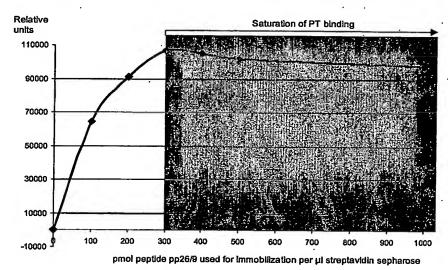


FIGURE 22

25/28 FIGURE 23







26/28

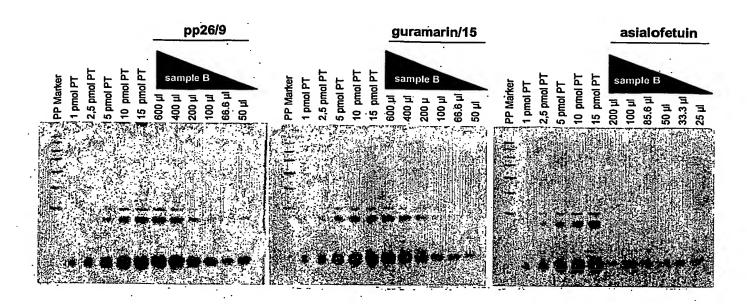
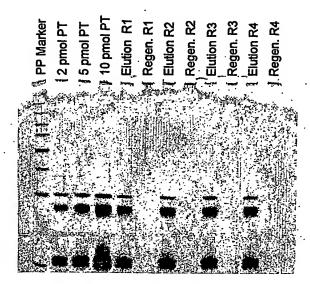


FIGURE 24



# gurmarin/15



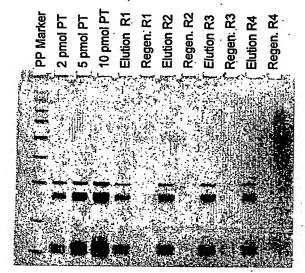


FIGURE 25



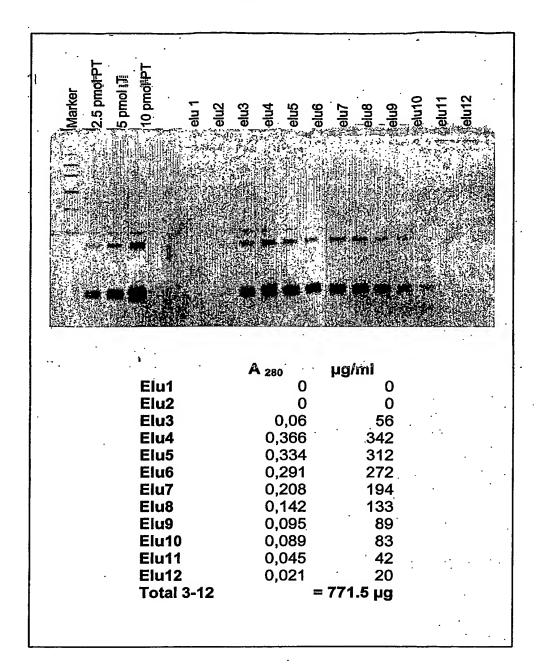


FIGURE 26

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